Clin. exp. Immunol. (1990) **81**, 459–465

Effects of interleukin-2 and interleukin-2-activated cells on *in vitro* myelopoiesis

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(Accepted for publication 3 April 1990)

SUMMARY

Lymphokine-activated killer (LAK) cells from human peripheral blood mononuclear cells cultured with recombinant interleukin-2 (IL-2) have been used clinically in adoptive immunotherapy for cancer patients. To study the influence of LAK cells and IL-2 on haematopoiesis, an *in vitro* assay system for colony formation of granulocyte-macrophage progenitor cells (GM-CFC) was used. LAK cells from cultures of either human peripheral blood (PB) or human bone marrow (BM) mononuclear cells were both inhibitory to allogeneic BM-derived GM-CFC. Inhibitory activity could be transferred with supernatants from co-cultures of LAK cells and BM targets, but also from the IL-2 activated PB- or BM-derived cells alone. The inhibitory activity from the initially non-cytotoxic/non-inhibitory BM population was rapidly induced by IL-2 activation, and preceded the generation of cytotoxic LAK cells in the culture. These experiments show that inhibition of haematopoietic progenitor cells by IL-2 is not dependent on generation of cytotoxic LAK cells, but rather the result of IL-2-induced cytokine production. We conclude that the synergistic action of interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) may contribute to inhibition, but that also other cytokines are responsible for the observed inhibition of BM-derived GM-CFC.

Keywords interleukin-2 haematopoiesis lymphokine-activated killer cells

INTRODUCTION

In vitro culture of peripheral blood mononuclear cells (PBMC) with the lymphokine IL-2 leads to induction of LAK cells, which are highly cytotoxic to various cultured and fresh tumour cells but without apparent cytotoxicity against normal cells (Grimm *et al.*, 1982; Lotzova, Savary & Herberman, 1986; Herberman, 1987). In experimental murine model systems, as well as in human therapeutic trials, the adoptive transfer of lymphokine-activated killer (LAK) cells and/or interleukin-2 (IL-2) have reduced tumour growth and metastatic spread, and prolonged survival of the host (Rosenberg *et al.*, 1985, 1987). In addition to these beneficial effects against certain tumours, several adverse effects have also been reported (Gately, Anderson & Hayes, 1988; Ettinghausen, *et al.*, 1987). These include haematological abnormalities such as anaemia, thrombocytopenia and eosinophilia, as well as non-haematological effects, manifested as the vascular leak syndrome (Gately *et al.*, 1988, Ettinghausen *et al.*, 1987).

Most of the cytolytic activity in the heterogeneous LAK population can be accounted for by cells with apparent identity to activated natural killer (NK) cells (Herberman, 1987; Phillips & Lanier, 1986). Previous studies on NK cells have shown that both murine (Kiessling *et al.*, 1977; Hansson *et al.*, 1988) and human NK cells (Hansson, Kiessling & Andersson, 1981; Hansson *et al.*, 1982; Mangan *et al.*, 1984; Degliantoni *et al.*, 1985b) can suppress allogeneic as well as autologous haematopoietic progenitor cells. Although the exact mechanism by which NK cells suppress *in vitro* colony formation of GM-CFC is not clear, lymphokines like interferon (IFN) and tumour necrosis factor (TNF) seem to play a major role (Degliantoni *et al.*, 1985a, 1985b). Activation of NK cells as well as T cells and monocytes by IL-2 has been shown to induce production of several lymphokines (Kasahara *et al.*, 1983a, 1983b; Ortaldo *et al.*, 1984; Procopio, Allavena & Ortaldo, 1985; Skettino *et al.*, 1988), all of which can be regulatory for haematopoietic progenitor cells (Skettino *et al.*, 1988; Lu *et al.*, 1986; Saito *et al.*, 1988). In view of the potential usefulness of LAK cells and lymphokine therapy in the treatment of malignancies, it seems important to elucidate the mechanisms of their adverse effects on normal haematopoietic cells.

In this study we have investigated the effects of LAK cells generated from PBMC or bone marrow (BM) mononuclear cells, as well as by IL-2 alone, on normal BM-derived *in vitro* myelopoiesis.

MATERIALS AND METHODS

Bone marrow and blood samples

Sternal marrow biopsies were obtained surgically from patients with no haematological disorders who were undergoing cardiac

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surgery at the Karolinska Hospital, Stockholm. The piece of marrow was collected in sterile phosphate-buffered saline (PBS), flushed repeatedly with PBS trough a needle to obtain a single-cell suspension.

Heparinized peripheral blood (PB) was obtained from healthy adults and diluted in PBS. Mononuclear cells from BM and PB were collected in the interface after centrifugation on a Ficoll–Hypaque density gradient (1·077 g/ml) at 400 *g* for 20 min, whereafter BM- and PB- mononuclear cells were washed, resuspended in medium (DMEM, GIBCO, Paisley, UK) and counted. These studies were approved of by the Local Ethics Committee at the Karolinska Institute.

Lymphokines and antibodies

Human recombinant IL-2 with specific activity 1×10^7 U/mg, was generously supplied by Drs J. Linna and J. Whitney, DuPont et Nemours, Glenolden, USA. Human recombinant IFN-γ (*Escherichia coli*-derived, specific activity 2×10^7 U/mg), human rec TNF-α (specific activity 6×10^7 U/mg), as well as a mouse monoclonal antibody (MoAb) against human TNF-α (neutralizing capacity 6000 U TNF/μg) were kindly provided by Dr G. R. Adolf, Vienna. The mouse MoAb GZ-4 specific for human IFN-γ (Lasky *et al.*, 1987) was a generous gift from Dr W. Berthold, FRG.

LAK cell cultures

For generation of IL-2-activated cells, PB or BM mononuclear cells were cultured at 1×10^6 cells/ml in RPMI medium containing 5% human $\mathbf{AB}^+$ serum, L-glutamine (300 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml) and 100 U/ml of human recombinant IL-2 (DuPont). PB-derived LAK cells (LAK_{PB}) were harvested and used after 3–4 days, and BM- derived LAK cells (LAK_{BM}) were cultured for 6–7 days (unless otherwise stated) in order to obtain optimal cytotoxic activity.

Percoll separation

Non-adherent PBMC were fractionated according to density over a seven-step discontinuous Percoll gradient (Pharmacia, Uppsala, Sweden) according to the method described by Timonen *et al.* (1982). Each fraction was collected separately, but for IL-2 activation fractions 2 and 3 were pooled and considered as low density (LD) cells; high density (HD) cells were harvested from fraction 6.

GM-CFC cultures

Mononuclear BM cells prepared as described above, or in some experiments after depletion of adherent cells by incubation on a plastic surface for 1 h at 37°C, were cultured in agarose for GM-CFC growth with a slight modification of the method by Pike & Robinson (1970). Briefly, a two-layer colony assay with human placenta conditioned media (HPCM, 20% v/v) in the bottom layer as colony-stimulating factor (CSF) and 1×10^5 BM cells in the upper layer was used. For assaying the effect of different supernatants derived from the LAK cultures, $2{\cdot}5 \times 10^5$ BM cells were pre-incubated in a total volume of 0·5 ml, containing 0·05 ml or 0·1 ml of the supernatant (thus never exceeding 20% v/v) for 16–18 h prior to the GM-CFC assay. Similarly, $2{\cdot}5 \times 10^5$ BM cells were pre-incubated in a total volume of 0·5 ml containing effector cells at various effector-to-target (E:T) ratios (ranging from 2:1 to 0·5:1). After the pre-incubation, the BM cells were washed and resuspended in 0·35% agar medium containing 20% fetal calf serum (FCS) and plated in duplicate plates.

Colonies containing > 40 cells were scored under an inverted microscope after 8 days of incubation at 37°C, in 7% CO_2. Data are expressed as % inhibition, by comparing the number of colonies in experimental culture plates with 4–8 control cultures (BM cells incubated in medium without addition of supernatant or effector cells).

Cytotoxicity assay

Effector cells suspensions (from LAK cultures or freshly harvested, non-adherent PBMC) were tested for cytotoxic activity against the erythroleukaemia cell line K562 in a standard 4-h ^{51}Cr release assay. Graded number of effector cells in 100 μl (giving E:T ratios ranging from 20:1 to 1:1) were seeded in triplicate in a V-bottomed 96-well microtitre plate. K562 cells labelled with ^{51}Cr for 60 min at 37°C and washed three times, were added at a concentration of 1×10^4 cells/well, giving a final total volume of 150 μl. After 4-h incubation, the plates were centrifuged at 100 *g* for 5 min and 75 μl supernatant were harvested from each well and counted in a gamma counter. Specific lysis was calculated according to the formula:

$$\frac{\text{Experimental} - \text{Spontaneous release}}{\text{Maximum} - \text{Spontaneous release}} \times 100.$$

Spontaneous release was determined from wells containing only medium and maximum by resuspending the target cells before harvesting.

TNF assay

TNF-α was assayed with an ELISA developed in our laboratory. Protein A-purified rabbit IgG anti-human TNF-α produced in our laboratory was diluted in a $NaHCO_3$, buffer, pH 9·0 to a concentration of 7 μg/ml for coating 96-well immunoassay plates (Nunc, Roskilde, Denmark) with 50 μl overnight at 4°C. After subsequent washing with PBS–0·01% Tween 20, non-specific binding of proteins to the plastic was blocked by a 1-h incubation with 100 μl PBS containing 0·5% gelatin, wherafter 50 μl of samples to be tested as well as serial dilutions of TNF-α standard were allowed to incubate for 1 h at room temperature. Bound TNF was detected after sequential incubation of biotinylated rabbit anti-TNF-α (5 μg/ml) and streptavidin-biotinylated horseradish peroxidase (HRP) (Amersham, UK) diluted to 1:1000. As a substrate, OPD (Dakopatts) was used and absorbance was measured at 492 nm in a Titertec Multiscan (Flow Laboratories, UK).

The detection limit, defined as the lowest concentration of TNF-α with absorbance significantly different from the negative controls was 5–10 U/ml (80–160 pg/ml) and no cross-reactivity with recombinant human IL-2, IFN-γ or IFN-α was observed.

IFN-γ assay

IFN-γ content of supernatants was determined with a sensitive ELISA assay, developed in our laboratory. The plates were first coated with 2 μg/ml of a affinity-purified goat anti-mouse IgG $(Fab)_2$ (Jackson Labs.) blocked with PBS/BSA, whereafter the IFN-γ-specific MoAb GZ4 was added (5 μg/ml). After incubation of the plates with samples to be tested and a serial dilution of IFN-γ standard for 1 h, a polyclonal rabbit anti-human IFN-γ antibody (pre-absorbed with normal human and mouse serum) was used (Interferon Sciences, NJ). HRP-conjugated

donkey anti-rabbit immunoglobulin (Amersham), also pre-absorbed with normal mouse and human serum was used as the last step. The plates were subsequently washed in between all steps and OPD substrate added as described for the TNF assay. The sensitivity of this assay was 3–4 U/ml.

RESULTS

LAK cells inhibit allogeneic GM-CFC

When LAK cells were assayed against normal haematopoietic progenitor cells in a non-cytolytic assay system (i.e. effects on GM colony formation) they were potent inhibitors of allogeneic GM-CFC growth (Table 1). To determine the level of NK-mediated inhibition (Hansson *et al.*, 1982; Degliantoni *et al.*, 1985b), freshly isolated PBMC were used in parallel in three of the experiments. As can be seen in Table 1, IL-2-activated LAK_{PB} and LAK_{BM} were more inhibitory than fresh PBMC (70·3% and 59·5%, respectively, *versus* 46·6% inhibition by PBMC).

Non-adherent PBMC were separated according to density on Percoll gradients (Timonen *et al.*, 1982) prior to IL-2 activation. After 3 days of culture in IL-2, LAK_{PB} generated from the LD fraction and those generated from the HD fraction were compared for inhibitory activity against allogeneic BM

Table 1. Inhibition of allogeneic GM-CFC by LAK cells

Source of effector cells*	Inhibition of GM-CFC (%)		
LAK_{PB} ($n=6$)	70·3±9·5	NS	$P<0·05$
LAK_{BM} ($n=4$)	59·5±16·3	NS	
PBMC ($n=3$)	46·3±4·5		
		Supernatants†	
HD-LAK_{PB} ($n=3$)‡	68·0±10·5	64·5±1·5	NS
LD-LAK_{PB} ($n=3$)	83·0±5·3	84·5±9·5	NS

* IL-2-activated LAK cells from PB, BM, or freshly isolated PBMC as described in Materials and Methods. Data shown are mean±s.d. from 3–6 experiments (n) at an E:T ratio of 1:1.

† Supernatants from 18-h co-cultures between the respective LAK cells and BM targets were assayed for inhibitory effect after pretreating BM cells with 20% (v/v) supernatant (mean from two experiments).

‡ Percoll fractionated PBMC as described in Materials and Methods. Data shown from an E:T ratio of 0·5:1.

NS, not significant ($P>0·05$)

Table 2. Cytotoxic activity against K562 by BMMC or PBMC after culture in IL-2

Days in culture (100 U/ml IL-2)	Lysis (%)*		
	E:T	BM-LAK	PB-LAK
4	10:1	10·1±0·3	57·2±2·3
	5:1	6·4±0·2	49·9±2·2
	2·5:1	4·5±1·1	36·4±3·3
6	10:1	30·6±2·1	50·1±6·0
	5:1	20·1±3·0	42·2±3·3
	2·5:1	11·8±2·0	26·5±1·5
9	10:1	25·8±3·4	38·4±5·0
	5:1	23·8±1·2	38·7±5·1
	2·5:1	21·8±3·0	30·0±4·1

* Lysis of K562 in a 4-h Cr release assay with the indicated E:T ratio.

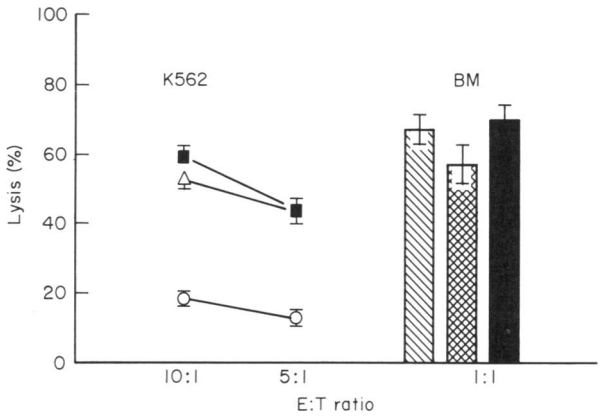

Fig. 1. Comparison between cytolytic activity against K562 and inhibitory activity against BM-derived GM-CFC. Effector cells were from 5-day LAK_{BM} (○, ▣); 7-day LAK_{BM} (△, ▨) and 3-day LAK_{PB} (■, ■). Number of colonies in control cultures was 66·5±2. No significant difference in inhibition between the effector cells ($P>0·05$).

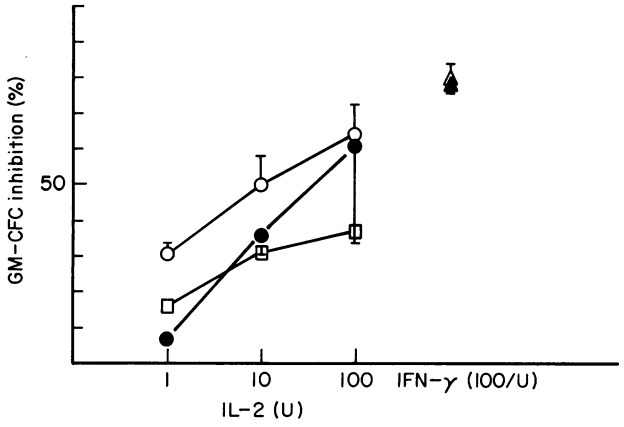

Fig. 2. Inhibitory effect of different doses of IL-2 on GM-CFC when added to the GM-CFC assay in the lower agar layer (□, one experiment); same layer as the BM cells (○, mean±s.d. from three experiments); or tested after pre-incubation and washed away prior to the GM-CFC assay, mean±s.d. from three experiments). In two of the assays, 100 U of IFN-γ present in the GM-CFC assay (△), or after preincubation (▲) was included. Control cultures from the respective experiments gave 173±4, 96±1·5, 92±4, 67±6, 30·5±3 and 88±6 colonies.

Table 3. Correlation of inhibitory activity with TNF-α and IFN-γ

BM culture	Harvest (day)	Inhibition of GM-CFC (%)* Exp. 1	Exp. 2	TNF-α (U/ml)	IFN-γ (U/ml)	Lysis of K562 (%)†
+IL-2	1	54±6	80±3	<10	95	ND
+IL-2	3	37±5	72±3	55	270	41·9±2·1
+IL-2/-IL-2‡	3‡	30±13§	65±2§	<10	90	39·1±1·6
+HPCM	3	2±1¶	7±1¶	<10	240	1·9±0·9

* Supernatants from the BM cultures were assayed for inhibitory activity by pre-treatment of fresh non-adherent BM cells for 18 h prior to the GM-CFC assay in two independent experiments. Control plates contained 109±19 and 130±2 GM colonies, respectively.

† When supernatant was harvested day 3, the remaining cells were kept in culture (without addition of IL-2 or HPCM) until day 7, when their lytic activity was assayed against K562. E:T 20:1 ratio is shown.

‡ This culture was washed free of IL-2 after 24 h, and further cultured without addition of IL-2 for 48 h.

§ Not significantly different from the IL-2 containing cultures, $P > 0.05$.

¶ Significantly different from the other cultures; $P > 0.01$ (Exp. 1), and $P < 0.001$ (Exp. 2).

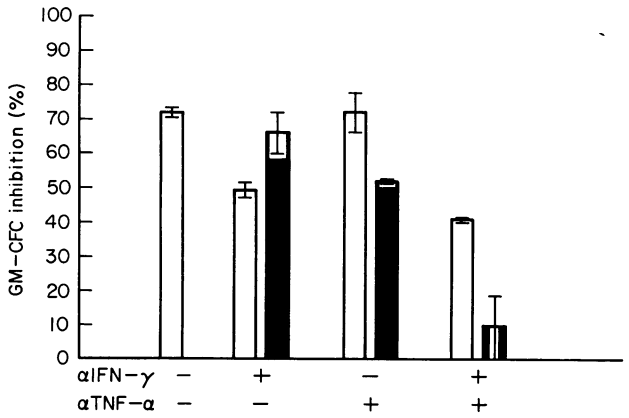

Fig. 3. GM-CFC inhibition by by supernatants derived from 3 days IL-2 activation of PBMC (□) or BMMC (■) assayed in two separate experiments with the addition of the indicated MoAbs for neutralization of IFN-γ, TNF-α or both. No. of colonies in control cultures (without addition of supernatant but the respective MoAbs) were: 111±2, 97·5±3 and 85·5±3 (exp. with supernatant from PBMC) and 110±0·5, 140±2 and 106·5±1·5 (exp. with supernatant from BMMC).

derived GM-CFC. Table 1 shows the mean % inhibition from three independent experiments at an E:T ratio of 0·5:1. Inhibition by LD- and HD-derived LAK_{PB} was similar (83%±5·3 *versus* 68%±10·5; $P > 0.05$; Table 1), although they differed with respect to the content of cells with $CD16^+$ phenotype (>32% and <5%, respectively).

Moreover, inhibitory activity could be transferred with the supernatants harvested from the 18-h co-culture between the respective effector cells and the BM target. Both LD- and HD-derived supernatants contained inhibitory activity (84·5%±9·5 and 64·5%±1·5, respectively; Table 1, mean from two experiments; $P > 0.05$).

LAK cells generated from human bone marrow mononuclear cells

Fresh BM cells obtained without aspiration, have no or very little NK activity (Hansson *et al.*, 1981; and data not shown). These mononuclear cells contain only a low number of $CD3^+$ cells (9·1%±2·8; $n=7$) and $CD16^+$ cells (5·1%±3·1, $n=5$). By culturing BMMC with IL-2, LAK_{BM} cells with cytolytic activity were generated. In our experience, optimal cytotoxic activity by LAK_{BM} cells against K562 required 6–7 days culture in IL-2, while LAK_{PB} activity peaked already at 3–4 days (Table 2). Control cultures of BMMC without IL-2 generated no cytotoxic activity against K562 (mean % lysis from five independent experiments at an E:T ratio of 10:1, 1·4%±1·4).

In spite of the relatively poor cytotoxicity against K562 by BM cells after only 5 days of culture in IL-2 (18·4±1·0% lysis at a 10:1 E:T ratio, Fig. 1), these effector cells produced a similar degree of GM-CFC inhibition as the highly cytotoxic 7-day LAK_{BM} and 3-days LAK_{PB} (57%±4, 67%±3, and 69%±4 inhibition, respectively; Fig. 1). Thus, inhibitory activity did not correlate with the degree of lytic activity by the effector cell population (Fig. 1). Since supernatants from E:T cell co-cultures contained inhibitory activity (Table 1), we investigated whether IL-2 activation of PBMC or BMMC, without triggering by allogeneic BM targets, could lead to release of cytokines inhibitory for colony formation. Supernatants harvested after 1 day or 3 days, of IL-2 activation of BMMC gave 64·6%±11 and 49·6%±15 inhibition, respectively (mean±s.d. from independent experiments). This suggested that soluble factors with ability to suppress allogeneic BM-derived GM-CFC were rapidly produced upon IL-2 activation.

Effect of IL-2 on the autologous BM-derived GM-CFC growth

Figure 2 shows that with doses ranging from 1 to 100 U/ml, IL-2 caused a dose-dependent suppression of the autologous colony growth. With the highest concentration (100 U/ml) of IL-2, 18 h pre-treatment resulted in a similar degree of inhibition as when IL-2 was present during the 8-days GM-CFC assay

(64% ± 8·5 and 61% ± 27 respectively, mean from three experiments, Fig. 2). For comparison, 100 U/ml of IFN-γ was used in parallel in two of the experiments (Fig. 2). Thus, it could be concluded that in contrast to the relatively slow generation of lytic LAK cells in the BM population (Table 2, Fig. 1), IL-2 rapidly induced an ability to suppress autologous myeloid progenitor cell growth. These experiments also showed that inhibition was not dependent on cell-to-cell contact between the IL-2-responsive cells and the progenitor target cells, since IL-2 could be seeded in any of the agar layers (where cells are prevented from movements; Fig. 2).

Lymphokine production by IL-2-activated PB and BM mononuclear cells

IL-2 has been shown to induce the production of IFN-γ and TNF by both T cells and NK cells (Kashara *et al.*, 1983b; Ortaldo *et al.*, 1984; Christmas, Meager & Moore, 1987); both of these lymphokines may act as inhibitors of haematopoiesis (Degliantoni *et al.*, 1985a; Lu *et al.*, 1986). We could also readily detect IFN-γ in the supernatants harvested from both LAK_{PB} and LAK_{BM} cultures, varying from as little as 20 U/ml up to 3000 U/ml, with the lower amounts always from the BM cultures (data not shown). TNF-α production by the same cultures was found to be more varying. While most supernatants from the BM cultures were negative for TNF-α (i.e. < 10 U/ml), and the occasional positive supernatants contained < 100 U/ml, the PB-derived supernatants contained up to 300 U/ml of TNF-α (data not shown).

Although there was no correlation between the content of IFN-γ and/or TNF-α and the degree of GM-CFC inhibition (Table 3; and data not shown), we have tried to neutralize the inhibitory activity with MoAbs to these lymphokines. As can be seen in Fig. 3, most of the inhibitory activity in BM-derived supernatants could be neutralized with antibodies to both lymphokines, but this was not the case with inhibitory supernatant from IL-2-activated PBMC (Fig. 3). We could not neutralize the inhibitory activity in any of the supernatants with antibodies to only one of the lymphokines (Fig. 3).

We also considered the possibility that part of the inhibitory activity in supernatants harvested early after IL-2 activation could be due to the exogenously added recombinant IL-2 remaining in the culture supernatant. Therefore, experiments were designed where BM cells were washed free of recombinant IL-2 after 1 day, then allowed additional 48-h incubation with IL-2-free medium. At the end of this incubation (3 days), supernatants were harvested and assayed for inhibitory activity, TNF-α and IFN-γ content (Table 3). No significant difference in inhibition could be seen comparing the supernatant from BM cultured in IL-2 for 3 days, and the BM culture washed free of IL-2 on day 1 and then harvested the third day (37% ± 5 *versus* 30% ± 13, respectively, in experiment 1, and 72% ± 3 *versus* 65% ± 2 in experiment 2; $P > 0{\cdot}05$). As expected, the supernatant collected after 1 day was slightly more inhibitory, most likely due to remaining recombinant IL-2, whereas BM cells cultured in the HPCM did not produce inhibitory activity (Table 3). After 7 days, both of the cultures which initially contained IL-2 had generated cytotoxic LAK cells, whereas HPCM cultures did not (Table 3).

The presence of IL-2 for 3 days resulted in higher production of both TNF-α and IFN-γ than did IL-2 activation for only 1 day (55 U and 270 U/ml, respectively, compared with < 10 U and 90 U/ml), although a similar degree of inhibition was seen (Table 3). These experiments clearly showed that inhibition of GM-CFC by IL-2 activation was not merely due to IFN-γ and TNF-α production. The synergistic action between these lymphokines may contribute to the inhibition, but induction of other inhibitory cytokines independent of TNF-α and IFN-γ must be considered as a possibility.

DISCUSSION

The present study shows that when human LAK cells are assayed for activity against normal cells in a non-cytolytic assay system (i.e. colony formation by BM-derived GM-CFC) they exert a strong inhibitory activity. Human IL-2-activated PBMC were recently shown to inhibit allogeneic BM-derived GM-CFC (Fujumori, Hara & Nagai, 1987), and we have extended this finding to IL-2-activated cells from BM, by showing they are suppressive for *in vitro* myelopoiesis from allogeneic and autologous BM.

Inhibition of GM-CFC by non-cultured PBMC has previously been shown to be mediated by cells with low density, LGL morphology, NK cell phenotype ($CD16^+$) and to correlate with their lytic ability (Hansson *et al.*, 1982; Degliantoni *et al.*, 1985b). This NK cell-mediated suppression is dependent upon cell-to-cell contact between the NK cells and the target cells as an initial step for NKCF-like inhibitory activity to be generated (Degliantoni *et al.*, 1985a, 1985b).

When IL-2 is used as the activating agent, both the CD16-enriched, non-adherent LD PBMC fraction from Percoll gradients and the CD16-depleted, HD fraction are inhibitory to a similar degree, and inhibitory activity can be transferred with supernatant from both of these effector cells. Even though the mechanisms for inhibitory activity and their nature may differ, depending on the effector cell fraction, these results indicate that the inhibitory activity observed in this study can not be accounted for only by NK cells.

In other experiments, we found that IL-2 activation of LD, HD, or total population of PBMC was sufficient, and that further triggering by the allogeneic BM target was not needed for generation of suppressive activity. This is seen also with the initially non-lytic BMMC, where activation with IL-2 rapidly (within 20 h) leads to generation of inhibitory activity acting on the allogeneic BM-derived GM-CFC as well as on the autologous progenitor cell growth (Table 3; Fig. 2).

This may seem somewhat contradictory to a previous report where IL-2 had no effect on GM-CFC growth, but rather selectively induced erythroid burst-promoting activity by PB-derived T and NK cells (Skettino *et al.*, 1988); however, in that study enriched progenitor cells from PB were used to assess effects on GM-CFC by IL-2. It is likely that even though PB- and BM-derived progenitors share certain surface markers and have a similar ability to produce colonies of various lineages, they may respond differently to certain regulatory mechanisms. The recent demonstration that PB-derived progenitor cells are resistant to IL-2-activated NK cells, while BM-derived GM progenitors are inhibited (Nagler *et al.*, 1988) futher supports this idea.

In the present study, supernatants containing IL-2-induced inhibitory activity are only tested on GM-CFC and not for effects on erythroid progenitors. However, inhibition of BM-derived BFU-e has previously been demonstrated as a result of

IL-2 activation by IL-2-responsive cells in the BM population (Burdach & Levitt 1987).

In our system optimal cytotoxicity from LAK_{BM} cultures requires 6–7 days culture, while inhibitory activity is rapidly induced by IL-2; therefore, the mechanism for inhibition seems disparate from cytotoxicity. We suggest that IL-2 acts as a rapid inducer of cytokines mediating most of the inhibitory activity. Likely candidates among the known inhibitory cytokines produced by the IL-2 activated BMMC or PBMC are TNF-α and IFN-γ (Degliantoni *et al.*, 1985a; Lu *et al.*, 1986). However, neither of these cytokines was found to be responsible for the inhibitory effect on GM-CFC growth; rather, an effect partly caused by synergism between the two, and most likely by other factor(s) not yet identified.

We are presently investigating the extent to which the different subpopulations of mononuclear cells in the BM contribute to the observed suppression. However, it should be pointed out that we have not used aspirated BM as a source of BMMC in order to avoid as much as possible the risk of 'contamination' of the BMMC with PB-derived cells. By FACS analysis, the BMMC used in this study contained $< 10\%$ $CD3^+$ cells ($9{\cdot}1\% \pm 2{\cdot}8$; $n = 7$), only $5{\cdot}1\% \pm 3{\cdot}1$ ($n = 5$) $CD16^+$ cells and no functionally mature NK cells (i.e. cytotoxic).

We conclude that the 'overall' *in vitro* effect of IL-2 on a heterogeneous BM population, similar to the population *in vivo*, leads to suppression of GM-CFC. Some of the severe haematological effects reported after *in vivo* administration of LAK cells and IL-2, such as anaemia, lymphopenia, thrombocytopenia and neutropenia (Ettinghausen *et al.*, 1987), can thus be accounted for. For the best therapeutic benefit of IL-2 and other lymphokines, it is essential to investigate further the complexity of their side effects on haematopoiesis. The ability of one lymphokine to induce production of others, and to synergize is complex and still poorly understood.

In a recent report Gately *et al.* (1988) showed that much of the toxic effects caused by IL-2 administration *in vivo* to mice is in fact due to the asialo $GM1^+$ LAK cell population. However, in agreement with our present findings these investigators concluded that not all the toxic effects could be accounted for by cells with the NK/LAK phenotype (Gately *et al.*, 1988).

We have shown in an *in vitro* system of culturing myeloid progenitor cells, that IL-2 activation leads to a rapid production of cytokines with suppressive effect on BM-derived GM-CFC.

ACKNOWLEDGMENTS

This work was supported by NIH grant CA 44882 and The Swedish Cancer Society. M.C. has been the recipient of a grant from the Basque government, Department of Health and Social Security.

The help from personnel at the Karolinska Hospital, Department of Thoracic surgery in providing us with bone marrow specimens is greatly acknowledged.

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